Conflict of interest

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Luca Valenti *
Università degli Studi di Milano, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Pad. Granelli, via F. Sforza 35, 20122 Milan, Italy
Tel.: +39 02 503 20278; fax: +39 02 503 20296
**E-mail address:* luca.valenti@unimi.itm

Massimo Colombo
Department of Internal Medicine, A.M. Migliavacca Center for Liver Disease, First Division of Gastroenterology, Università degli Studi di Milano, Fondazione IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, Milan, Italy

Silvia Fargion
Department of Internal Medicine, A.M. Migliavacca Center for Liver Disease, First Division of Gastroenterology, Università degli Studi di Milano, Fondazione IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, Milan, Italy

Reply to: "Modulation of the effect of *PNPLA3* I148M mutation on steatosis and liver damage by alcohol intake in patients with chronic hepatitis C"

***PNPLA3* rs738409 and fibrosis progression in chronic hepatitis C – There is more to it than just fat!**

To the Editor:

The Hepatology community has seen a substantial rise in publications reporting data on genetic risk factors conferring risk to fibrosis progression in non-alcoholic, alcoholic, and chronic hepatitis C-related liver diseases. For long, hypothesis-driven, often single-centre genetic case control studies have reported associations of certain candidate genes with fibrosis/cirrhosis which could not be replicated. With genome-wide association studies (GWAS) becoming a standard tool in translational research, novel candidate genes enter the stage which have been identified by systematic screening experiments [1]. GWAS make use of a hypothesis-free, or rather – hypothesis-generating approach, and have the potential to uncover genetic risk factors which had not been considered based on our previous pathophysiological understanding. A striking example for such revelation is the gene coding for patatin-like phospholipase domain containing-3 (*PNPLA3*; adiponutrin) of which a polymorphic variant (rs738409 G >C) was found associated with liver fat content in a landmark GWAS [2], and subsequently with progression of non-alcoholic fatty liver disease (NAFLD) [3], and alcoholic liver disease (ALD) [4,5] by means of candidate case control studies. The role of *PNPLA3* rs738409 as a risk factor for progressive fibrosis can now be extended to chronic hepatitis C. Only in 2011, four studies presented data on the role of *PNPLA3* rs738409 G which unanimously confirm that carriage of at least one allele increases the risk of advanced fibrosis and cirrhosis [6–9] (Table 1). Our own data indicate that this association is particularly evident in hepatitis C virus-infected individuals who regularly drink alcohol as opposed to abstainers [6]. Regarding the latter, *PNPLA3* rs738409 G appears to modulate fat storage in the liver by a yet unknown mechanism, possibly by interaction of *PNPLA3* with viral epitopes. Indeed, from the data presented in published studies, it becomes clear that *PNPLA3* rs738409 G neither aggravates steatosis nor fibrosis in patients with genotype 3, supporting the hypothesis that mostly genotype 1-specific interactions with *PNPLA3* are involved.

In this issue of the *Journal*, Valenti and coworkers have undertaken a pooled analysis of our [6] and their data [7] after stratifying their patients into "at-risk" drinkers (daily alcohol consumption $\geqslant$20 g) and abstainers [10]. Hereby, they confirm that carriage of *PNPLA3* rs738409 G is a risk factor for steatosis in abstainers, but not in at risk drinkers, possibly due to a confounding effect on steatosis by concomitant alcohol consumption. In addition, by increasing the numbers of total cases and controls,

Table 1. Genetic candidate gene association studies on the role of *PNPLA3* rs738409 G as a risk factor for fibrosis and cirrhosis.

Author [Ref]	Patients (n)	End points	Subgroup analysis	Results			
					OR	95% CI	*p* value
Müller *et al.*, [6]	Steatosis (442) Cirrhosis (605)	Steatosis Cirrhosis	Abstainers "At-risk" drinkers	Association with • steatosis in abstainers • cirrhosis in "at-risk" drinkers	 12.61 4.77	 1.48-107.7 1.39-6.38	 0.021 0.013
Valenti *et al.*, [7]	Two independent cohorts (325/494)	Steatosis Cirrhosis HCC Therapy response	None	Association with • steatosis • fibrosis/cirrhosis • therapy response • HCC	 1.9 1.47 0.63 2.61	 1.4-2.7 1.2-19 0.4-0.8 1.3-3.6	 <0.001 0.002 0.006 0.002
Cai *et al.*, [8]	626	Steatosis	Genotype 3 Genotype non-3	Association with • steatosis but only in viral genotype non-3	 1.88	 1.57-2.25	 <0.001
Trepo *et al.*, [9]	537	Steatosis Fibrosis Fibrosis progression Therapy response	None	Association with • steatosis • fibrosis • fibrosis progression	 2.55 3.13 2.64	 1.08-6.03 1.50-6.51 1.22-5.67	 0.034 0.002 0.013

they convincingly show that *PNPLA3* rs738409 G is also a risk factor for cirrhosis in abstainers, with an additional increase of the odds ratio (OR) in at risk drinkers. Of note, a subgroup analysis of our latest data [9], confirmed these observations [6,10]. Again, only abstainers were at risk of steatosis (OR = 4.52 95% confidence interval [CI] 1.32–15.48, $p = 0.016$). Moreover, *PNPLA3* rs738409 G remained associated with advanced fibrosis in abstainers (OR = 2.68 95% CI 1.18–6.06, $p = 0.018$), but still its impact on fibrosis was stronger in "at-risk" drinkers (OR = 10.34 95% CI 1.15–93.16, $p = 0.037$). In our view, these analyses provide a perfect example of host-environment interactions in which the risk of developing cirrhosis increases in a "dose-dependent" manner with the number of risk factors present (*PNPLA3* rs738409 G, hepatitis C virus, and alcohol).

Together, data from these four independent studies deliver strong evidence for a significant role of *PNPLA3* rs738409 for progressive liver disease not only in NAFLD and ALD, but also in CHC. What needs to be elucidated now is to decipher the cross-talk between PNPLA3 (and its genetic variation) and fibrosis-related intracellular signalling. Fat accumulation may do harm in hepatitis C infected subjects – but as we know from NAFLD, it requires a "second hit" to cause inflammation and fibrosis.

Conflict of interest

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Felix Stickel *
Department of Internal Medicine I, University Hospital Schleswig-Holstein, Kiel, Germany
Department of Visceral Surgery and Medicine, Institute of Clinical Pharmacology and Visceral Research, Inselspital, University of Bern, Murtenstr. 35, 3010 Bern, Switzerland
**E-mail address:* felix.stickel@ikp.unibe.ch

Eric Trépo
Christophe Moreno
Department of Gastroenterology, Hepatopancreatology and Digestive Oncology, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium
Laboratory of Experimental Gastroenterology, Université Libre de Bruxelles, Brussels, Belgium

Jochen Hampe
Department of Internal Medicine I, University Hospital Schleswig-Holstein, Kiel, Germany